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13. ABSTRACT *(Maximum 200 Words)*

The presence of p53 mutations in tumor tissue have been hypothesized to represent a "fingerprint" of environmental carcinogens. As a first step in testing this hypothesis in breast cancer in humans, we are evaluating whether risk for women with tumors that show p53 protein overexpression (p53+) assessed by immunohistochemistry in relation to certain environmental exposures, such as hormone replacement therapy, alcohol use, cigarette smoking, DDT levels in blood, or polycyclic aromatic hydrocarbons (PAH-DNA adducts), is higher than risk among women with tumors that show no p53 protein overexpression (p53-), as compared with population-based controls. For this molecular epidemiology project, archived tumor tissue is being retrieved for the case participants of the NIH-funded parent study, the Long Island Breast Cancer Study Project. The retrieved archived tumor tissue is being cut and prepared to establish a tumor bank that can be linked to already collected risk factor data and stored samples of blood and urine. Two prepared slides per subject are being utilized for the p53 immunohistochemical assays. The lab data from the molecular epidemiology component will be coupled with the risk factor data on the respondents of the parent study to perform statistical analyses to evaluate the hypothesis of the molecular epidemiology component.

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p53, Environmental Risk Factors and Breast Cancer:
A Population-Based Study

(4) INTRODUCTION

The presence of *p53* mutations in tumor tissue have been hypothesized to represent a "fingerprint" of environmental carcinogens. As a first step in testing this hypothesis in breast cancer in humans, we are evaluating whether risk for women with tumors that show *p53* protein overexpression (*p53*+) assessed by immunohistochemistry in relation to certain environmental exposures, such as hormone replacement therapy, alcohol use, cigarette smoking, DDT levels in blood, or polycyclic aromatic hydrocarbons (PAH-DNA adducts) in blood, is higher than risk among women with tumors that show no *p53* protein overexpression (*p53*-), as compared with population-based controls.

This study draws upon an ongoing population-based, case-control study, with the specific aims of determining whether breast cancer risk is related to blood levels of organochlorine compounds (including DDT, DDE, PCBs, and chlordane) or polycyclic aromatic hydrocarbons (PAH-DNA adducts). For the parent study, interviews were completed with 1,508 case women and 1,556 control women. For the offspring molecular epidemiology component, archived paraffin-embedded tumor tissue blocks are being retrieved for the 1,453 case women with signed medical record release forms from the 33 participating hospitals. The retrieved archived blocks are being prepared and cut at Columbia University for immunostaining for *p53* protein overexpression, and for storage for future molecular epidemiology studies. Laboratory results from the proposed study will be combined with the interview data, and laboratory results from blood samples, which are collected and analyzed as part of the parent study. The purpose of these combined statistical analyses is to determine whether the risk of *p53*-positive breast cancer in relation to certain environmental exposures (including hormone replacement therapy, alcohol use, cigarette smoking, DDT/DDE, PAH-DNA adducts, and others) is higher than risk for *p53*-negative breast cancer, as compared with population-based controls. Results from this study will help to identify a subgroup of women that may have tumors that are associated with environmental exposures. Future research efforts could then focus on this subgroup to identify signature *p53* mutations for the carcinogens.

(5) BODY

The grant application described the workscope of the study as follows. This three-year project includes four components: (1) retrieval of 1,453 paraffin-embedded tissue blocks from a population-based sample of breast cancer cases; (2) preparation of slides from the archived tissue for the planned assays, and to bank for future studies; (3) evaluation for evidence of *p53* overexpression in the archival tumor tissue by immunohistochemistry; and (4) estimation of the odds ratios for *p53* positive breast cancer in relation to environmental factors, information which is already being collected by the investigators. Most of the components of the study are to be conducted simultaneously, as described below.

Task 1. Retrieval of Paraffin-embedded Blocks, Months 1 - 32.

A. Request paraffin-embedded blocks from the appropriate hospitals.

B. After cutting slides from each block, return the block to the appropriate hospital.

C. Track on personal computer the collection and return of each block.

Task 2. Slide Preparation, Months 2 - 32.

A. Cut and prepare slides from the retrieved archived tissue according to the study protocol.

B. Bank tissue for future studies.

Task 3. Laboratory Analyses, Months 2 - 33.

A. Prepare slides from paraffin-embedded blocks.

B. Determine adequacy of tissue sample.

C. Evaluate tumor tissue for evidence of *p53* by immunohistochemistry.

D. Interpret and record immunohistochemical results.

Task 4. Data Entry and Statistical Analyses, Months 2-36.

A. Enter laboratory results into a SAS file on personal computer.

B. Merge (1) laboratory data that will be collected in the proposed study, and (2) the case and control data on risk factors that is already being collected and computerized by the investigators.

C. Determine, using SAS on personal computer, the odds ratios for breast cancer by *p53* status in relation to (1) hormone replacement therapy, (2) alcohol, (3) cigarette smoking, (4) DDT levels, (5) PAH-DNA adducts, and (6) other risk factors for breast cancer.

As of the end of year 2, all tasks have been initiated as follows. In our undertaking of these research activities, no unusual problems have been encountered to date.

- **Task 1.** Of the 1,508 case women who participated in the parent study (the case-control interview of the Long Island Breast Cancer Study Project), 1,453 signed a medical record release form, which gives us access to her archived pathology specimens. In year 1, initiation of the block retrieval was delayed due to delays in the field work of the parent study. For the parent study, medical records were collected from each of the 35 participating hospitals at the completion of the field work. In an effort to reduce the volunteer labor of the participating hospitals, we delayed contacting hospitals for block retrieval (Task 1). Once retrieval was underway, we decided to request the archived tumor tissue blocks in waves, in a further effort to prevent the participating hospitals from being overburdened.

  To date (at the end of year 2), blocks have been requested from all 35 participating hospitals for 1331 of the 1,453 subjects (91.6%). Fifteen of the 35 hospitals (42.9%) have provided us with 100% of the requested materials; seven (20.0%) have provided between 90-99% of the blocks requested from their institution; five (14.3%) have provided between 80-89% of the blocks requested; one (2.9%) has provided us with 70-79% and one (2.9%) has provided us with 50%. Four institutions have yet to provide us with any material; three of these four have agreed to provide us with the blocks and are working on our request, whereas we are in intensive negotiations with one remaining institution to reach an agreement that the blocks will be provided. Thus, blocks have been successfully retrieved for 784 case participants (54.0%). This is one of the largest banks of archived breast cancer tissue that also has corresponding data on risk factors.

- **Tasks 2 and 3.** For the laboratory component, services (slide preparation and laboratory assays of p53), have been completed on 703 case women (89.7%) using the following methods. The paraffin blocks from each case participant are used to generate 15- 5 micron and 10-10 micron thick 5-micron slides. Selected sections are baked at 60°C for 30 minutes. Twenty-three sections are banked (protected from light and stored at -20°C) for future molecular studies. Because such little tissue material is needed for the assay and the diagnoses of breast cancer are so recent, few cases are expected to have a tissue sample that is inadequate or too small. However, in no instance is the block exhausted. Instead, because the goal is to return one-half of the archived tumor tissue to the lending institution, the final quantity of slides cut is based on the tissue available. Thus in some instances, less than 25 slides are cut, prepared, and banked.

For the ongoing study, slides are being used to evaluate for evidence of *p53* protein overexpression by immunohistochemical staining utilizing antibodies with high sensitivity for these oncogenes in paraffin-embedded tissues. The details of the laboratory methods being used for the assessment of p53 expression for immunohistochemistry have been published previously (Gammon 1999).

- **Task 4.** We have developed the program for the data entry component. The statistical analyses will begin towards the end of year 3 once all blocks have been retrieved, cut, stained, and interpreted.

(6) KEY RESEARCH ACCOMPLISHMENTS

- **Initiation of Tumor Bank.** Our research efforts include initiation of a bank of archived tumor issue among a population-based sample of breast cancer cases who were residents of Long Island and who were diagnosed with a first primary invasive or in situ breast cancer between August 1, 1996, and July 31, 1997, and who participated in a comprehensive case-control interview, donated a 40 ml blood sample, and a casual urine sample. The study also included interviews and donation of blood and urine from a population-based sample of control women from the same geographic area. Because there were no age restrictions for eligibility in the parent case-control study, this newly initiated tissue bank may yield one of the largest archived banks based on a population-based study of older women. Thus, this archived tumor bank will be extremely valuable because of its unique link to risk factor data as well as results of laboratory assays based on other types of biologic specimens, and the unusual age range of the study subjects.

  As described above, archived tumor blocks of breast cancer tissue have been successfully retrieved for 784 case participants (54.0%). Thus, even before the completion of the study, this is one of the largest banks of archived breast cancer tissue that also has corresponding data on risk factors for breast cancer.

(7) REPORTABLE OUTCOMES

- **Publications.** None in year 2 related to this project.

- **Presentations.** Presentations made by the Principal Investigator over the past year in which the design and conduct of the ongoing study were discussed are listed below.

2000 Diet and Cancer (NUTR 362), "Polycyclic aromatic hydrocarbons, p53 mutations, and breast cancer," University of North Carolina School of Public Health, Chapel Hill, NC, Spring

"Understanding the Peer-Review Process For Scientific Publications," Long Island Breast Cancer Network, Omni Center, Uniondale, NY, January

1999 "Polycyclic Aromatic Hydrocarbons and the Risk of Breast Cancer: Scientific Rationale and Issues in Data Interpretation," Long Island Breast Cancer Network, American Cancer Society of Long Island, Hauppauge, NY, November

"Long Island Breast Cancer Study Project," Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, October

(8) CONCLUSIONS

The goal of the ongoing molecular epidemiology component of the Long Island Breast Cancer Study Project are to evaluate the relation between p53 expression measured by immunohistochemistry and environmental risk factors for breast cancer. This project is at the completion of the second year of three years of funding from the U.S. Army. Three of the components of the study protocol (block retrieval, slide preparation, and data entry) have been initiated as planned; lab assays for p53 are scheduled to be undertaken at the beginning of year 3. Although implementation of the field activities was delayed in year 1, due to unforeseen delays in the NIH-funded parent study on which this ongoing study is based, research efforts are underway and close to the target goals. No unusual problems in any of the four study components have been encountered to date.

(9) REFERENCES

Gammon MD, Hibshoosh H, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Cigarette smoking and other risk factors in relation to *p*53 protein expression in breast cancer among young women. Cancer Epidemiology, Biomarkers and Prevention 1999;8:255-263.

(10) APPENDICES

None.